NON-TECHNICAL SUMMARY

Implications of therapeutic inhibition of the complement system on infectious and non-infectious inflammatory diseases

Project duration
5 years 0 months

Project purpose
- (a) Basic research

Key words
Complement System, Kidney injury, Bacterial infection, Fungal infection, Immunotherapy

Animal types | Life stages
--- | ---
Mice | adult, juvenile, neonate, pregnant, embryo

Retrospective assessment
The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits
Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

**What is the aim of this project?**

This project aims to validate the utility and efficacy of a novel therapeutic technology to reduce kidney injury in some diseases. We will also look for possible side effects that may result from using this technology like an increase in the risk of infection with microbial pathogens and how to reduce this risk.

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

The complement system (a group of circulating proteins in the blood) is an important component of the immune system that participates in recognition and killing of invading microbial organisms. Activation of the complement system also triggers the inflammatory response. Uncontrolled activation can cause tissue injury and the development of some inflammatory diseases. Development of inhibitors that block the complement system in the blood will open a new avenue for novel approaches and strategies for the treatment of inflammatory disorders. Unfortunately, the use of complement inhibitors may increase the risk of microbial infection in patients and hence it is important to address the fundamental interactions between the host immune system and different pathogens during the course of active infection. Understanding the underlying mechanisms and pathways involved in the immune defence will also help to develop novel approaches for the treatment of bacterial and fungal infections.

**What outputs do you think you will see at the end of this project?**

Our research in the next five years will provide novel findings that will help to identify novel therapeutic approaches that will help to alleviate complement-mediated tissue injury especially in case of haemolytic uremic syndrome (HUS) and lupus nephritis. In addition, our results will provide a better understanding of how the immune system behaves towards invading pathogens especially in case of complement deficiency. This will help to develop new strategies for the treatment of bacterial and fungal infection. We always publish our new findings in high impact factor scientific journals to disseminate it to the scientific community. Unsuccessful approaches will also be highlighted in our publications and discussed with our collaborators.

**What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?**

In the short term the outputs will be communicated to academics working in the field of immunology, where we will assess both the benefits and the harmful effects results from complement activation and
address the delicate balance between complement activation and down-regulation in health and disease.

In the long term we will introduce a new and well-defined study in the field of complement immunology during infection that will help to better understand how the immune system fights microbes, and how to avoid the harmful effects resulting from uncontrolled activation of the immune system. This work might also introduce new therapeutic approaches that will help to reduce kidney injury in lupus nephritis and HUS and improve the outcome in these patients.

**How will you maximise the outputs of your work?**

Our group has a close collaboration with experienced Senior Scientists in our field, who have established track records in mouse models of experimentally induced infectious disease, stretching over several decades whom I meet on a regular basis to discuss results and future experiments. A complete survey on the published data has already been done and we know exactly what is published in our area of study. We will use the most refined and the most reliable models for our infection studies as well as for HUS and lupus nephritis models. We always publish our new findings in high impact factor scientific journals to introduce it to the scientific community. Unsuccessful approaches will also be discussed with our collaborators and will be mentioned in our publications. Sharing of animal tissue from our well-designed experiment with our collaborators will help to maximise the benefits.

**Species and numbers of animals expected to be used**

- Mice: 2000

**Predicted harms**

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

In our mouse models of microbial infections:

- Mice will be infected with bacteria or fungi via intranasal or oral administration as well as by injection.

- Mice will be observed for disease progression.
- Immunomodulatory and/or complement inhibitors therapeutics could be injected at any time either before or after the infection.

- Blood samples will be taken at different time points post-infection to assess the bacterial load in blood to avoid any complications that may increase the severity of disease progression beyond the expected severity limit.

- Our experience shows that the infection study in protocol 1 will last for 3 weeks while other infection models in protocol 2 will last for 7 to 14 days.

In case of lupus nephritis model:

- Mice at the age of 14 weeks will start to develop signs and symptoms of renal injury.

- At that age we will treat mice with immunomodulatory and/or complement therapeutics that will reduce complement-mediated kidney injury.

- In some experiments, mice will be treated with complement inhibitors therapeutics/immunomodulatory compounds at earlier ages (10-12 weeks).

- This study will continue for 8 to 10 weeks

All animal experiments will be randomised and people who are assessing the animal will not be aware of whether they are assessing control or treated mice.

**Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.**

In mouse models of infection, mice will show signs and symptoms of moderate severity limit including reduction in their activity within the cage and decrease in body weight. Animals will be closely observed as frequently as necessary and at least every 6 hours to ensure that these symptoms would not last for more than 12 hours. If these signs persist for over 12 hours or if an animal begins to deteriorate at any time, it will be immediately killed.

Lupus nephritis is inflammation of the kidney due to an anauto-immune disease known as systemic lupus erythematosus. With lupus, the body’s immune system targets its own body tissues including kidney causing kidney inflammation and kidney injury. In a mouse model of lupus nephritis, mice will start to show some complications depending on the stage of the disease progression and the age of the mouse. From 14-16 weeks old, mice will show symptoms of mild disease severity such as a decrease in body weight (5-10%) and a slight increase in some biological markers that reflect how kidneys are functioning such as blood urea nitrogen (BUN) and creatinine levels in the blood. Older mice will show a moderate decrease in body weight (10-15%) and significantly elevated levels of BUN and creatinine levels.
Because the strain of mice that we will use in this protocol spontaneously develop auto-immune disorders, mice will also show some other side effects such as enlarged lymph nodes and skin lesions that will be considered during the course of our experiment.

Animals will be humanely killed by once they reach predetermined end-point for this protocol.

**Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per species)?**

1. In protocols 1 we will use 500 adult mice at moderate severity limit
2. In protocols 2 we will use 1000 adult mice at moderate severity limit
3. In protocols 3 we will use 500 adult mice at moderate severity limit
4. In protocol 4 we are expecting mild severity limit (All animal).

**What will happen to the animals at the end of the study?**

- Killed

**Replacement**

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

**Why do you need to use animals to achieve the aim of your project?**

We will study how the immune response mediates tissue injury in some auto-immune diseases such as Haemolytic Uremic Syndrome (HUS) and lupus nephritis. In addition, we need to study the complex interaction between the innate immune response and different pathogens. These processes involve a complex interaction between immune cells, plasma proteins, the blood vessel walls (vascular endothelium) and organ-specific cells that cannot be modeled using ex vivo (using animal tissues in cell culture) or in vitro (in test tubes rather than in animals) systems.

**What was your strategy for searching for non-animal alternatives?**

Prior to animal experimentation extensive in vitro studies using different techniques to assess the ability of different proteins from the immune system to bind bacteria such as (ELISA and FACS analysis
techniques). Opsonophagocytosis assays (engulfment of bacteria by white blood cells isolated from human blood) will be performed to identify which strains/serotypes of the pathogen will stimulate the targeted pathway of the complement system. Assessment of the functional activities of the complement inhibitors will be completed first in-vitro using ELISA and FACS analysis to evaluate the specificity and efficacy of these complement inhibitors. We considered the possibility of limiting the infection experiments to ex-vivo studies using blood from human volunteers as a source of complement and cells. Ex-vivo studies do not reproduce the course of the infection in multi-organ model (i.e in animals)

Why were they not suitable?

It is necessary to study microbial interaction with the immune system in a whole mammalian organism to appreciate the impact of medical treatments on:

a) The spread of the bacteria between different organs in the body
b) Tissue pathology in various organs
c) The effects of the progressive escalation of immune responses

In addition, we need to assess the degree of kidney injury as a result of uncontrolled complement activation in some autoimmune disorders such as lupus nephritis and HUS. This study requires the tissue to be exposed to different complement components and recognition molecules for a prolonged period of time to achieve tissue injury and there is no available ex-vivo model for such complications.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our long-term experience in animal work, using good experimental design helped us to give the estimated numbers of animals that we will need to use in each experiment. We also consulted with experienced scientists in our field and within our group. In addition, we consulted a departmental statistician who gave us advice and help whenever needed. Our previously similar animal models published in high impact factor scientific journals were also a useful guide to estimate the number of animals that will be used in this project.
What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Whenever possible we perform preliminary studies using smaller numbers of animals. We also try to adjust our work to use only one control group that can be used for different experiments. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of the results. To calculate the smallest number of animals that we can use, calculations based on advanced statistics and mathematics in addition to other published results were taken into considerations. Online tools such as experimental design assistant (EDA) from NC3Rs website were used to perform sample size calculations. To increase the quality, reproducibility and translatability of our animal studies we will follow PREPARE guidelines (Adrian et al., 2018). Animal tissues from different experiments will be kept frozen and/or embedded in paraffin blocks that can be used later if more tissue analysis is required.

What other measures apart from good experimental design will you use to minimise numbers?

We always run pilot experiments using a small number of animals to assess the feasibility of the study.

In some experiments we will use bacterial toxins instead of using the bacteria. This will help to minimise the side effects where mice will receive only the calculated dose of the bacterial toxins that show the symptoms and the signs of disease without exposing animals to the bacteria. We will run pilot experiments to test the potency of the bacterial toxins and so large stock of bacterial toxins will be purchased to avoid repeating this step every time we buy a new batch.

We would not start any animal work in large groups until we have preliminary positive and encouraging data. Tissues from pilot experiments and major experiments will be kept frozen or fixed in paraffin blocks for future use. For genetically modified mice, we will follow a breeding protocol that gives us only our gene-targeted mice by breeding deficient mice whenever possible to avoid the production of unnecessary mice.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?
All animal models in this study are designed to cause less pain, stress and suffering. All the necessary procedures will be taken to achieve high levels of welfare to the animal starting from good husbandry and animal care before starting the experimental procedures. We will use the most refined mouse model of infection for each pathogen. During the experimental procedures in the infection study (Protocols 1 and 2) all requirements will be taken to cause less stress and minimal harm to the animal. E.g. In case of i.n (intra-nasal) infection we will use light anaesthesia to reduce animal stress and maximise the volume being delivered. We will use small gauge and fine needles to minimise trauma in the case of intra-venous and intra-peritoneal drug administration. Mice will be monitored for any unexpected signs and symptoms of disease progression to avoid exceeding the allowed severity limit. Mice will be euthanised at early end points to avoid exceeding the allowed severity limit (moderate).

In the lupus nephritis (kidney injury) model (Protocol 3), we will use a specific strain of mice that spontaneously develop kidney disorders and validate our complement inhibitor therapeutics to prevent the onset of disease progression. Lupus prone mice will survive normally without any complications until they reach the age of 14 weeks when they start to develop signs and symptoms of lupus nephritis and kidney injury. These symptoms include; elevated levels of creatinine and blood urea nitrogen, increased albumin and protein levels in urine. We will not inject any compounds that could accelerate kidney injury but ameliorate or prevent inflammation and tissue injury. We will use therapeutic compounds that are expected to minimise the kidney injury and improve the renal function.

**Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?**

We need to study the immune response towards bacterial infection and how the immune response might cause tissue injury as a result of uncontrolled activation of the complement system. This will require animals to be exposed to the insults for a relatively long time (days or weeks) and this cannot be done in anaesthetised mice. In addition, using immature life stage of animals is not a good choice to study the immune response, especially since immature developmental stages of animals are likely to have an immature immune system and therefore will provide results that cannot be extrapolated to reflect the responses expected in mature animals.

**What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harm) to the animals?**

Mice will be closely monitored for any sign of disease progression. Mash (soaked mouse pellets which are easier to access and eat) will be used during our studies if needed to minimise weight loss. Any pain or stress will be diagnosed as early as possible and animals will be culled by humane methods (schedule 1 methods) if necessary. A proper environment including shelter and a comfortable resting area will be provided to the animals. All clinical signs and manifestations will be scored in scoring sheets. These scoring sheets will be kept and discussed after each experiment to refine our procedures.
What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Our group has an excellent reputation in the field of complement immunology and we keep ourselves updated with all new and novel experimental designs and models in our field. This includes non-animal models. All newly published data in our field are discussed internally within our group to enrich our knowledge and that could help to find novel experimental designs or other non-animal models that could help in our study. In addition, all our experiments will be conducted in a way that allows us to publish our results according to the ARRIVE guidelines. we will follow all guidelines provided by LASA, PREPARE and NC3Rs.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

By following the updates on NC3Rs and NORECOPA (websites as well as additional guidance literature from Laboratory Animal Science Association (LASA). Related events such as conference and symposium will be attended.

Explain the choice of species and the related life stages

We will use adult wild type and adult complement deficient mice that lack one or more of the essential components of the immune system. Using these mice will give a clear overview of how the absence of these immune components will affect the infectivity and pathogenicity of different pathogens included in this project. We will also use adult MRL-Ipr/Ipr (Lupus) mouse, a strain that is known to spontaneously develop lupus nephritis and kidney injury after 14 weeks of birth. This model will help us to validate how inhibition of the complement system will improve kidney function in such mouse model.

We will use adult mice in our models of diseases because the immune response at that stage captures the essential traits of many bacterial infections and tissue injury in humans.